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BIOSYSTEMATICS OF AEDES (NEOMELANICONION)

Annual Report

Thomas J. Zavortink

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19. Abstract

The objective of the "Biosystematics of Aedes (Neomelaniconion)" project is to produce a modern taxonomic monograph of the aedine subgenus Neomelaniconion. Comparative morphological taxonomic procedures will be emphasized. Characteristics from both sexes and all stages of the life cycle will be studied.

During the third contract year, an ultrafreezer was acquired. Additional specimens of Neomelaniconion were obtained through loans from the British Museum (Natural History) and the United States National Museum and through field work by staff and cooperators in Senegal and Central African Republic. A total of 120 field collections was made. Over 400 egg clutches obtained in the field were flooded in the laboratory and progeny series were reared. Approximately 4500 adult mosquitoes, 2600 immature mosquitoes, and 114 male genitalia were prepared for study. Approximately 750 adult mosquitoes were frozen for electrophoretic study. All specimens of Neomelaniconion acquired by the project to date have been provisionally identified, and 29 species are represented. A partial or complete set of the preliminary drawings of the larva, pupa, and male genitalia of ten species of Neomelaniconion was completed. Neomelaniconion larvae may be reared easily in alkaline water to which sand and dried leaves of woody angiosperm plants are added. Study of type specimens has shown that Aedes albothorax, pallida, and punctocostalis are senior synonyms of circumluteolus, mcintoshi, and monotrichus, respectively, and that Aedes maculicosta is a valid species. Taxonomic study has shown that the subgenus is correctly divided into two major groups, the forest and savanna groups. The forest group is best represented in forested parts of west and central Africa and includes numerous undescribed species. Species in this group may be distinguished easily by larval and male genitalic characters, but not at all or only with difficulty by female characters. The savanna group includes fewer species than the forest group, but the group is more widespread in the Ethiopian Region, occurring in regions of savanna and grassland in northern, eastern, and southern Africa. This group includes Aedes bolensis and one possibly new species. Species of this group are distinguished by small but reliable differences in females and male genitalia. Aedes lineatopennis does not occur in Africa; only the related mcintoshi occurs there, but this species should properly be called pallida. Aedes circumluteolus and mcintoshi are very widespread in the Ethiopian Region.

Contents

Statement of the Problem

The goal of the project "Biosystematics of Aedes (Neomelaniconion)" is to produce a modern taxonomic monograph of this subgenus of mosquitoes. This group, which is primarily Ethiopian in distribution, has not been studied carefully, and so its species are poorly known. The absence of basic information on the number of species and on how to distinguish them severely hampers the acquisition and reporting of biological information about these mosquitoes. The result is that the distribution, bionomics, and disease vector potential of the different species remain unknown or uncertain.

Species of Neomelaniconion are believed to be involved in both the inter-epizootic maintenance and transmission of Rift Valley fever virus. A complete understanding of the natural history of this virus is not possible without better knowledge of these mosquitoes.

Background

As it is presently understood, the subgenus Neomelaniconion includes 28 nominal species, 24 of which are considered to be valid taxonomic species or subspecies (1-3). All except one of the currently recognized species are restricted to the Ethiopian Region. The exception is Aedes lineatopennis (Ludlow), which is widespread in the Oriental and Australian regions.

The existing taxonomy of the subgenus Neomelaniconion dates back to Edwards's treatment of the group under its former name, Banksinella Theobald, in his catalog of the family Culicidae (4) and in his volume on Mosquitoes of the Ethiopian Region (5). Edwards's studies were based almost entirely upon adult mosquitoes, and characteristics of the immature stages were not considered. In the many decades since Edwards's brief taxonomic treatments of Neomelaniconion, there has been no comprehensive study of the group. Several additional species have been described (3, 6-10), immatures of a few species have been partially described or illustrated (7, 9, 11-17), one nominal species has been transferred to the subgenus (18), and two nominal species have been removed (19).

In the absence of a comprehensive study of Neomelaniconion, the subgenus remains poorly and inadequately known. The immature stages, in particular, have been neglected. They have never been used to help define the species of the group or to help place these species into a natural classification. In fact, to this day the immatures

of nearly half the species of Neomelaniconion are unknown, and for those species in which they are known, they have been described and illustrated very superficially. The complete larval and pupal chaetotaxy has not been studied for a single species. Available keys to adults (5, 9, 15) and larvae (11, 15) of Neomelaniconion are inadequate because they treat only a portion of the species now known or treat only the species of a restricted region.

Numerous arboviruses have been isolated from species of Neomelaniconion (20). The virus that causes Rift Valley fever, an important disease of domestic animals and humans in Africa and a potential international disease problem (21), is the most important of these. This virus has been isolated from field populations of three or more species of Neomelaniconion: circumluteolus (Theobald) in South Africa (22) and Uganda (23); lineatopennis in Kenya (24), South Africa (25), and Zimbabwe (26); palpalis (Newstead) in Central African Republic (27); and possibly luteolateralis (Theobald) in South Africa (28). Laboratory experiments have shown that the virus can be transmitted horizontally by yet another species of Neomelaniconion, unidentatus McIntosh (29). Studies of Rift Valley fever in Kenya have provided evidence that lineatopennis is a reservoir for the virus between epizootics, transmitting it transovarially from generation to generation (30). The identity of the Neomelaniconion species reported as lineatopennis in all of these studies is in doubt; in Kenya the species if probably the recently described mcintoshi Huang (3), but in South Africa it may be an undescribed sibling species in this complex. The fact that Rift Valley fever virus has been isolated from several species of Neomelaniconion and is known to be transmitted horizontally or vertically by some of these mosquitoes underscores the importance of obtaining basic information on the systematics and biology of species of Neomelaniconion, for such information is critical to a complete understanding of the natural history of Rift Valley fever virus.

Approach to the Problem

A modern systematic study of Neomelaniconion, utilizing morphological characteristics from both sexes and all stages in the life cycle, will be undertaken in order to determine the number of species in the subgenus, the most reliable means of distinguishing these species from each other, the existence and nature of intraspecific variation, the geographic distribution of the species, and the evolutionary relationships of the species. The results of this study will be published in a monograph that will include: taxonomic descriptions of species and groups of species;

identification keys for all stages in the life cycle; detailed drawings of the larva, pupa, and male genitalia of each species and of the adult morphology for selected species; photographs of eggs; information on type specimens; synonymies; discussions of diagnostic characters, variation, and relationships; summaries of bionomics and medical importance; data on geographical distribution of the species, including lists of specimens examined and maps; and a bibliography.

Although the historically important specimens of Neomelaniconion currently held in museums will be examined, the bulk of the specimens studied will be collected specifically for the project. The collection, rearing, and preservation of material and the recording of field data will follow the procedures developed for the "Mosquitos of Middle America" project (31). Emphasis will be placed on collecting adult females from which eggs for progeny rearings can be obtained and on collecting the immature stages so they can be reared individually. Both progeny rearings and individual rearings associate the stages of a species, and progeny rearing associate the sexes unequivocally. Specimens collected in the field or borrowed from museums will be prepared for study using standard laboratory procedures for mosquitoes, in general following the methods of Belkin (19). Classical, comparative morphological taxonomic procedures will be emphasized, as outlined for mosquito systematics by Belkin (19) and Zavortink (32). The form of presentation and terminology used in the final monograph will follow Belkin (19) and Zavortink (33-35) in large part.

The initial phases of research on the "Biosystematics of Aedes (Neomelaniconion)" project must, out of necessity, emphasize training of staff, development of field and laboratory techniques, field work to collect and rear specimens, and laboratory work to prepare the specimens for critical study.

Results and Discussion

Accomplishments related to the goal of producing a monograph of the subgenus Neomelaniconion that were completed during the third contract year of the project "Biosystematics of Aedes (Neomelaniconion)" are described below.

FACILITIES

A 10.3 cubic foot ultrafreezer was purchased for the project. This makes it possible to freeze and store specimens of Neomelaniconion for electrophoretic studies as they are being reared in the laboratory.

STAFF

The following full-time and part-time staff were supported by the contract during the third year:

Thomas J. Zavortink, Principal Investigator (50% time)
Sandra S. Shanks, Taxonomic Research Specialist (100% time)
Mary Ann Tenorio, Scientific Illustrator (Piecework)

COOPERATORS

The following individuals contributed to the "Biosystematics of Aedes (Neomelaniconion)" project during the third contract year:

Y. Boulvert, Director, ORSTOM Center, Bangui, Central African Republic, provided vehicles and a chauffeur during the trip to Central African Republic, and authorized use of the ORSTOM field station at Bozo.

Jean-Paul Cornet, Institut Pasteur, Dakar, Senegal, facilitated the collecting of Neomelaniconion in Senegal and provided laboratory space.

Thomas P. Gargan, U.S.A. Medical Research Unit - Kenya, sent specimens of Neomelaniconion from Kenya.

Bernard Geoffroy, ORSTOM, Montpellier, France, made arrangements with Institut Pasteur and ORSTOM Center in Central African Republic and Senegal for joint field studies with the Principal Investigator, and helped with the collection of Neomelaniconion in those countries.

Alan Georges, Director, Institut Pasteur, Bangui, Central African Republic, provided laboratory facilities, lodging, field equipment, and field collectors during the trip to Central African Republic.

Jean-Paul Gonzales, Institut Pasteur, Dakar, Senegal, facilitated the collecting of Neomelaniconion in Senegal and arranged to have mosquito eggs shipped to San Francisco.

Scott Gordon, USAMRIID, Fort Detrick, Frederick, Maryland, provided some field equipment in Senegal and helped with the collection of Neomelaniconion.

G. Nguerekata Mandata, High Commissioner of Research, Central African Republic, gave authorization to travel and conduct research on mosquitoes in Central African Republic.

Philippe Salles, Societie Industrial Central Africa, Mbaiki, Central African Republic, provided lodging and working space at the SICA headquarters and gave permission to travel and collect mosquitoes in logging areas under his control.

Sabastian Talec, Bobenga Bouchia, Central African Republic, provided lodging, meals, and working space at his coffee plantation.

Mark Wilson, Institut Pasteur, Dakar, Senegal, facilitated the collecting of mosquitoes in Senegal by providing field equipment and assistance with logistics.

ACQUISITION OF SPECIMENS

Loans from Museums. - The primary types of eight nominal species of Neomelaniconion were borrowed from the British Museum (Natural History) and the United States National Museum. Specimens of lineatopennis from the Oriental Region were received from the United States National Museum.

Gifts of Specimens. - Reared specimens of two species of Neomelaniconion, circumluteolus and mcintoshi, from Kenya were received from Thomas P. Gargan. Among the specimens is a gynandromorph of mcintoshi.

Collecting and Rearing. - Mosquitoes were collected by the Principal Investigator and cooperators in Senegal during August and September 1988 (23 collections) and in Central African Republic during September and October 1988 (97 collections). Only a very few adult Neomelaniconion were collected in Senegal. These represented three species: bolensis Edwards, jamoti Hamon & Rickenbach, and mcintoshi. Only seven vials of eggs were obtained from female Neomelaniconion. All the egg clutches were very small and many of the eggs did not hatch; as a consequence, very few progeny were reared in the laboratory. Quite in contrast to the situation in Senegal, great numbers of adult Neomelaniconion of both sexes were collected in Central African Republic. The following 12 species were obtained in that country: bergerardi Pajot & Geoffroy, circumluteolus, crassiforceps Edwards, fuscinervis (Edwards), jamoti, palpalis, pogonurus Edwards, taeniarostris (Theobald), and undescribed species numbers 1, 3, 5, and 8. A total of 442 vials with eggs of Neomelaniconion was obtained from females collected in Central African Republic, and progenies of all species except pogonurus have been reared in the laboratory.

The difficulty of rearing Neomelaniconion larvae in the laboratory related in previous Annual Reports for the "Biosystematics of Aedes (Neomelaniconion)" project has been largely overcome during the third contract year. Further experimentation focusing on waters of different hydrogen-ion concentrations, on different larval foods, on different types of leaves or stems that are added to the rearing pans, and on other "additives" to the rearing medium has finally resulted in a successful rearing method. With this method, larval survival is high and the adults reared are large and robust and do not shrivel when killed and pinned for taxonomic purposes. It may be possible to streamline the rearing method developed by deleting some of the additives, but experimentation has not progressed to that point, and it may never do so. The Principal Investigator's attitude about the rearing procedure at this point in time is that "If it works, don't fix it."

Although there is inconclusive evidence about the value of some of the additives used in the rearing method developed, the Principal Investigator believes strongly that there is adequate evidence to support the following conclusions about rearing Neomelaniconion larvae: 1) larvae of all species of Neomelaniconion survive better, grow faster, and become larger if they are reared in alkaline water, and, for species in the forest group of Neomelaniconion, it is essential that the rearing water be alkaline; 2) larvae of all species of Neomelaniconion benefit from the addition of dried leaves of angiosperm trees or shrubs (or extracts of such leaves) to the rearing medium, and, for species of the forest group, it is essential that dried leaves or their extracts be used; 3) larvae of all species of Neomelaniconion benefit from the addition of very small amounts of sand to the rearing containers; and 4) powdered tropical fish food (TetraMin brand) is superior to liver powder or yeast as a larval food.

The success of the rearing method that has been developed is due, in large part, to using water from a local natural lake. The importance of this water is that it is alkaline, pH 8.4, and is strongly buffered, so that its hydrogen-ion concentration scarcely changes when larvae are reared in it or when leaves or larval food are added to it. Even after seven to nine days, at which time all larvae have usually pupated, the lake water used in the rearing pans is still alkaline, its pH having dropped only to 8.2. After the lake water is collected, it is filtered through cloth to remove larger planktonic organisms, sterilized in an autoclave, and stored in glass bottles for use in the laboratory.

The complete egg-hatching and larval rearing procedure now being used in the laboratory for all species of Neomelaniconion is as follows: 1) the oviposition vials with eggs are placed in a humid chamber at 25°C for 24 to 48 hours; 2) the oviposition vials with eggs are filled with a solution of 0.1% nutrient both in sterilized lake water in the late afternoon (1600-1700 hours) and incubated at 25°C; 3) early the following morning (0800-0900 hours), small larvae are transferred from the oviposition vials to plastic rearing cups with 350 ml sterilized lake water, a small piece (5 to 9 cm long) of dried grass leaf (Hordeum species, family Gramineae), a short section (3 to 4 cm long) of dried tule stem (Scirpus species, family Cyperaceae), a small piece (5 to 15 cm^2) of dried bigleaf maple leaf (Acer macrophyllum, family Aceraceae), and a very small amount (0.1 to 0.2 gm) of sterile sand, and reared with aeration at 25°C; 4) on the next (second) morning, a single drop of Avitron Liquid Vitamin Supplement (for pet animals) is added; 5) on the second or third day, and usually every day thereafter, larvae are fed a slurry of powdered TetraMin tropical fish food; 6) if larvae are numerous (more than 20), they are moved to

rearing pans that hold a larger volume of water when they have developed to large second instar to small fourth instar size; at this time the pieces of grass and tule may be discarded, but an additional piece of maple leaf and more sand are added if larvae are moved to a pan; 7) if larvae have been fed adequately, pupation usually starts on the sixth or seventh day, depending upon the species, and is completed within two more days.

The dried grass leaf and dried tule stem used in step 3 and the vitamins used in step 4 are not essential for larval development, but they do seem to enhance larval survival. A weak solution of Lipton tea may be used in place of the piece of bigleaf maple leaf used in step 3. However, California black oak (Quercus kelloggi, family Fagaceae) leaves are not a suitable substitute for the bigleaf maple leaves because they turn the water acid, and Norway maple (Acer platanoides) leaves are not usable because an oily substance that seems to be toxic to larvae leaches from them. While some leaf matter is essential for survival of most larvae in the forest group of Neomelaniconion, too much leaf can be as deleterious as too little. Liver powder may replace the TetraMin as the larval food in step 5. Larvae develop faster (about one day less to pupation) when fed liver powder, but they do not become as large. Baker's yeast is not a suitable larval food for species in the forest group because it makes the water acid, but it can be used for species in the savanna group, which seem to be less affected by hydrogen-ion concentration.

PREPARATION OF SPECIMENS FOR STUDY

All mosquitoes collected in South Africa during the second contract year or reared in the laboratory at the University of San Francisco from eggs obtained in South Africa have been prepared for study and labeled. All field-collected specimens obtained in Senegal and Central African Republic in August to October 1988 have been prepared for study. All egg clutches obtained in Senegal have been flooded, the larvae reared, and the specimens prepared for study. Most of the egg clutches obtained in Central African Republic have been flooded and the larvae reared. And, most of these reared specimens have been prepared for study. All specimens from Senegal and Central African Republic must yet be labeled with printed locality labels.

During the third contract year, approximately 4500 adult mosquitoes were mounted on points; approximately 2600 microscope slides of immature mosquitoes (whole larvae and larval and pupal exuviae) were prepared; 114 microscope slides of male genitalia were prepared; and approximately 750 adult mosquitoes were frozen for possible use in

electrophoretic studies.

IDENTIFICATION

Specimens of Neomelaniconion borrowed from museums or received as a gift during the third contract year and specimens in the collection at the Institut Pasteur in Dakar, Senegal, were identified. Five species were represented in this material. These species and the countries in which they were collected are:

Aedes (Neomelaniconion)
- bolensis Senegal
- circumluteolus Kenya, Senegal
- jamoti Senegal
- lineatopennis Philippines
- mcintoshi Kenya, Senegal

During the third contract year, 14 species of Neomelaniconion were collected for the project. These species and their geographic origins are:

Aedes (Neomelaniconion)
- bergerardi Central African Republic
- bolensis Senegal
- circumluteolus Central African Republic
- crassiforceps Central African Republic
- fuscinervis Central African Republic
- jamoti Central African Republic, Senegal
- mcintoshi Senegal
- palpalis Central African Republic
- pogonurus Central African Republic
- taeniarostris Central African Republic
- undescribed species #1 Central African Republic
- undescribed species #3 Central African Republic
- undescribed species #5 Central African Republic
- undescribed species #8 Central African Republic

During the third contract year, almost no time was spent identifying mosquitoes other than Neomelaniconion collected in Africa. However, final determinations of species of Aedes collected in ground pools in Kenya and Zambia in fall 1986 were made so the identifications could be sent to cooperators in those countries. The species reported to these cooperators were:

Aedes (Aedimorphus)
- aboventralis (Theobald) Zambia
- cumminsii (Theobald) Kenya, Zambia
- dalzieli (Theobald) Zambia
- dentatus (Theobald) Kenya
- eritreae Lewis Kenya

fowleri (Charmoy) Zambia
hirsutus (Theobald) Kenya, Zambia
?minutus (Theobald) Kenya
ochraceus (Theobald) Kenya, Zambia
quasiunivittatus (Theobald) Kenya, Zambia
vittatus (Bigot) Kenya

Aedes (Mucidus)
mucidus (Karsch) Zambia
sudanensis (Theobald) Kenya, Zambia

A very few species collected in Central African Republic and Senegal were provisionally identified on the basis of external adult characters only, as they were being reared. These species are:

Aedes (Aedimorphus)
argenteopunctatus (Theobald) Senegal
ochraceus (Theobald) Senegal

Aedes (Diceromyia)
furcifer (Edwards) Senegal

Aedes (Finlaya)
ingrami Edwards Central African Republic

Aedes (Stegomyia)
africanus (Theobald) Central African Republic
apicoargenteus (Theobald) Central African Republic
luteocephalus (Newstead) Senegal

Culiseta (Theomyia)
fraseri (Edwards) Central African Republic

ILLUSTRATION

During the third contract year preliminary pencil drawings were completed for the larvae of eight species of Neomelaniconion, the pupae of seven species, and the male genitalia of ten species. These drawings must be checked and corrected for the modal number of branches in each seta in a sample of specimens before they can be inked.

Scanning electron micrographs of the eggs of nine species of Neomelaniconion were prepared during the year. Scanning electron micrographs of the eggs of 18 species of Neomelaniconion have been prepared to date.

TAXONOMIC STUDY

During the third contract year the holotypes or lectotypes of eight nominal species of Aedes (Neomelaniconion) were examined. These were: albothorax (Theobald), bolensis, fuscinervis, maculicosta Edwards, mcintoshi, monotrichus Edwards, pallida (Theobald), and punctocostalis (Theobald). Examination of these types has confirmed the suspected synonymy of monotrichus with punctocostalis reported in the

Second Annual Report, and has revealed two additional unsuspected synonymies. The names albothorax and pallida are senior synonyms of circumluteolus and mcintoshi, respectively. The "Eastern form" of albothorax recognized by Edwards (5) is indeed a distinct, but unnamed, species. The Principal Investigator believes that it would be very confusing to synonymize circumluteolus with albothorax, in essence transferring the name albothorax from a relatively obscure East African species to the common, widespread, and important species now called circumluteolus. Therefore, a proposal to suppress the specific name albothorax for the purposes of the Law of Priority will be submitted to the International Commission on Zoological Nomenclature. This will permit the continued use of the name circumluteolus for the species that has borne this name in the literature since 1908. The specifically distinct "Eastern form" of albothorax will, of course, require description and naming. At this time it is referred to as undescribed species number 9. Since the name mcintoshi was proposed only recently (1985) and is not yet in widespread use, the Principal Investigator believes synonymization of this name under pallida is appropriate and will not cause confusion. Examination of the type of maculicosta shows that this species is not a synonym of carteri Edwards, as stated in the current world catalog of mosquitoes (1), or of punctocostalis, as suspected by the Principal Investigator and reported in the Second Annual Report. Aedes maculicosta is, instead, a valid species, apparently the one collected by the Principal Investigator in Ivory Coast in 1987 and reported in the Second Annual Report as ?maculicosta. Examination of the types of bolensis and fuscinervis confirms the current use of these names. Aedes bolensis is, however, in the savanna group of Neomelaniconion rather than the forest group.

Examination of specimens from Senegal in the collection of the Institut Pasteur in Dakar has contributed significantly to understanding what species of Neomelaniconion occur in Senegal and Gambia. Species known to occur in these countries are bolensis, circumluteolus, jamoti, mcintoshi, and undescribed species number 6. The records of fuscinervis males and palpalis females recorded from Gambia by Mattingly (36) are erroneous; the specimens examined by Mattingly are actually the two sexes of undescribed species 6. Aedes albothorax, described from Gambia, is the same species as circumluteolus.

Many valuable specimens of Neomelaniconion were collected and reared for the project in Central African Republic during the third contract year. Included among these are progeny series of six species not previously available: bergerardi, crassiforceps, palpalis, and undescribed species numbers 3, 5, and 8. Aedes bergerardi, previously known from males

only, is a very distinct species of Neomelaniconion. Aedes crassiforceps was previously known from males and a single female provisionally considered to be this species by Edwards (5). Progeny reared from females collected in Central African Republic indicate that Edwards erred in associating this female with crassiforceps. The female of crassiforceps is actually very similar to that of palpalis except for its simple hind claws and incompletely banded proboscis. And, male progeny reared from females similar to the one provisionally considered to be crassiforceps by Edwards are jamoti. Aedes palpalis, the type species of Neomelaniconion, is amply distinct in all stages from other species of the forest group. This species if far more variable than described by Edwards (5), and, in fact, most female progeny reared from mothers collected in Central African Republic cannot be identified as palpalis in the key given by Edwards (5) because they possess a dorsal submedian light-scaled mark on the hind tibia. Aedes palpalis was the most common species of Neomelaniconion collected in Central African Republic. It was particularly abundant in areas of humid forest south and southwest of Bangui. Undescribed species 3 was known previously from a single male collected in Ivory Coast in 1987. The affinities of this species are not obvious now that all stages are known. The adult female is almost identical to that of taeniarostris, but the larva and male genitalia are very different. Undescribed species 5 and 8 are related to palpalis and carteri, respectively. The male genitalia of undescribed species 5 are, in some respects, intermediate between those of palpalis and crassiforceps.

By the end of the third contract year, the Principal Investigator had examined at least one stage of all but two of the currently accepted species of Neomelaniconion. The exceptions are ellinorae Edwards and flavimargo Edwards, species which at present are known from only a limited area along the coast of Kenya. On the basis of the specimens studied to date, the Principal Investigator agrees with Edwards's (5) division of the subgenus Neomelaniconion into distinct forest and savanna groups. This division is supported by characteristics of the adult (tergal markings, length of cercus, presence or absence of hypostigial scales), male genitalia (dentition of aedeagus), and egg (shape, sculpturing).

The species of the forest group of Neomelaniconion are, as the group name implies, largely restricted to areas of humid forest or dense gallery forest. As a consequence, the group is best represented in the wetter portions of Africa, particularly West and Central Africa. The group is represented in East Africa, though, by ellinorae and flavimargo, which are perhaps associated with humid coastal

forests. As noted in the section on Collecting and Rearing, it is almost impossible to rear species of the forest group in the laboratory without adding leaf matter from woody angiosperms to the rearing water. The species included in the forest group are : bequaerti Wolfs, bergerardi, carteri, crassiforceps, ellinorae, flavimargo, fuscinervis, jamoti, maculicosta, palpalis, pogonurus, punctocostalis, taeniarostris, and undescribed species numbers 1, 2, 3, 4, 5, 6, and 8. In this group of Neomelaniconion the species are well-differentiated from each other in larval and male genitalic characters. However, adult females are often difficult or impossible to identify, in part because of the subtle differences between many of the species, in part because many of the specific characteristics are quantitative rather than qualitative, and in part because of the great range of variation displayed by several of the species. Characteristics of the male genitalia and larva, when known, suggest a large species group comprised of bequaerti, carteri, crassiforceps, palpalis, pogonurus, and undescribed species 5 and 8. Characteristics of the male genitalia and, to a lesser extent, adult females suggest two smaller species groups: fuscinervis and undescribed species 2 and 6; and maculicosta, punctocostalis, and undescribed species 4. Species without obvious affinities are bergerardi, jamoti, taeniarostris, and undescribed species 1 and 3. Aedes ellinorae and flavimargo have not been studied.

The Ethiopian species of the savanna group of Neomelaniconion are associated with savannas and grasslands in the drier regions of northern, eastern, and southern Africa. African species of the group are: albicosta (Edwards), aurovenatus Worth, bolensis, circumluteolus, luridus McIntosh, luteolateralis (Theobald), mcintoshi, unidentatus, and undescribed species 7 and 9. Aedes lineatopennis, the only Neomelaniconion species distributed outside Africa, is also a member of the savanna group. Species of the savanna group are best separated from each other by slight differences in coloration of the adult females and small but reliable differences in the male genitalia. Data available at this time indicate that albicosta, circumluteolus, and undescribed species 9 form a species group. Groupings of the other species are unclear. Aedes bolensis, luridus, and unidentatus seem isolated from all the other species and from each other. The remaining named species, aurovenatus, lineatopennis, luteolateralis, and mcintoshi, share some colorational characteristics in the adults, but, beyond that, there is little to unite them. Aedes bolensis was included in the forest group by Edwards (5), who knew only the male sex, but it actually belongs to the savanna group. Females of this species can be very similar to females of mcintoshi, but they can be distinguished reliably by their toothed hind claws. The

status of undescribed species 7 has not been resolved yet; instead of being a distinct species, it may represent only an isolated, marginal population of mcintoshi adapted to the colder climate of the highveld in South Africa.

The Principal Investigator concurs with Huang (3) that the African "lineatopennis" is specifically distinct from the true lineatopennis of the Orient. Huang (3) named the African species mcintoshi, but, as noted earlier in this section, this name is a junior synonym of pallida. When she described mcintoshi, Huang (3) left open the possibility that the true lineatopennis might also occur in Africa. However, it does not. With the possible exception of undescribed species number 7 noted in the previous paragraph, all African specimens of "lineatopennis" examined by the Principal Investigator are mcintoshi. This species is one of the most widespread Neomelaniconion in Africa, specimens having been seen from: Senegal, Nigeria, Sudan, Ethiopia, Zaire, Kenya, Tanzania, Angola, Zambia, Bechuanaland, Zimbabwe, and South Africa. The species may occur throughout regions of savanna, steppe, and grassland vegetation in sub-Saharan Africa.

The species of Neomelaniconion from which the greatest number of arboviruses has been isolated is circumluteolus (20). This species is also very widespread in Africa, and is known to occur in: Senegal, Gambia, Ivory Coast, Ghana, Nigeria, Central African Republic, Sudan, Ethiopia, Zaire, Uganda, Kenya, Rwanda, Zambia, Malawi, Mozambique, and South Africa. In Ivory Coast and Central African Republic, this species is abundant in regions of savanna, but it occurs also in smaller numbers in some areas of primary humid forest. Again, as noted earlier in this section, circumluteolus is a junior synonym of albothorax.

Hennig 86, a computer program for phylogenetic analysis, was obtained during the contract year. Several preliminary analyses of Neomelaniconion species, using adult characters only, and using Aedes (Aedimorphus) vexans (Meigen) as an outgroup, have been completed. Although the Principal Investigator has strong reservations about the utility of cladistic analysis to ascertain relationships among species, it is hoped that this program will be helpful in this regard.

Conclusions

The following are concluded as a result of the third year's activities of the project:

1. The rearing of Neomelaniconion larvae is facilitated by the use of alkaline water, leaves of woody angiosperm plants, sand, and powdered Tetramin tropical fish food.

2. The specific names circumluteolus, mcintoshi, and monotrichus are junior synonyms of albothorax, pallida, and punctocostalis, respectively.

3. Aedes maculicosta is a valid species.

4. Five species of Neomelaniconion are known to occur in Senegal and/or Gambia; these are bolensis, circumluteolus, jamoti, mcintoshi, and undescribed species number 6.

5. Characteristics of adults, male genitalia, and eggs support division of Neomelaniconion into distinct forest and savanna groups.

6. Species of the forest group of Neomelaniconion are well-differentiated from each other in larval and male genitalic characters, but not in female characters.

7. The forest group of Neomelaniconion includes several undescribed species.

8. Species of the savanna group of Neomelaniconion are separated from each other by small but reliable differences in adult female and male genitalic characters.

9. Aedes bolensis belongs to the savanna group. Females of this species can be very difficult to separate from those of mcintoshi.

10. The savanna group of Neomelaniconion includes one possibly new species and one recognized species that requires renaming.

11. Aedes mcintoshi is specifically distinct from lineatopennis, and it is the only one of these two species that occurs in Africa.

12. Aedes mcintoshi and circumluteolus are the most widespread species of Neomelaniconion in Africa. Both occur from Senegal to Ethiopia, and from Ethiopia to South Africa.

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